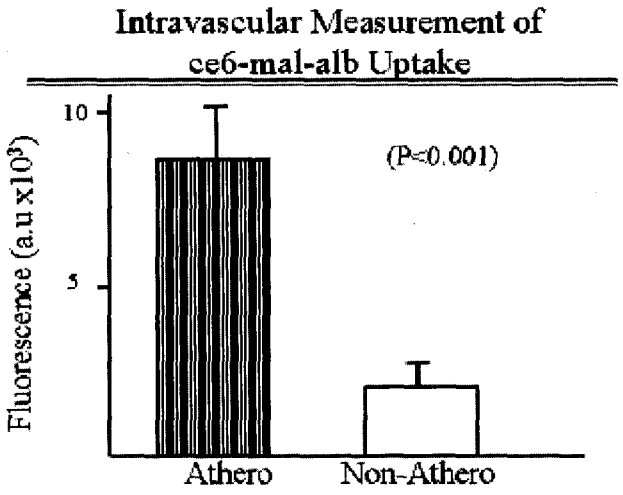
1. There is a dramatic downregulation of α-actin and vimentin microfilaments in atherosclerotic plaque.
2. OxLDL markedly downregulates VSMC α-actin and vimentin expression via a PPARγ and p38 MAPK independent pathway. OxLDL regulation of VSMC cytoskeletal proteins may play a critical role in atherogenesis through alterations in cell motility, differentiation and growth.

1200-138 Intravascular Detection of Inflammed Atherosclerotic Plaques With a Novel Macrophage-Targeted Fluorescent Photodynamic Compound

Ahmed Tawakol, Alan Fischman, Henry Gewirtz, James E. Muller, Thomas Brady, Michael Hamblin, Mass General Hospital, Boston, MA

Background: Vulnerable plaques contain abundant macrophages. We developed a novel photodynamic agent, chlorin$_{e6}$ conjugated with maleylated albumin, (ce6-mal-alb) that concentrates in macrophage-rich plaques and has a high fluorescent yield. As such, we tested hypothesis that experimental atherosclerotic lesions (ATHERO) can be detected using ce6-mal-alb and an intravascular fluorescence spectroscopy catheter.
Methods: ATHERO were induced in New Zealand rabbits by infradiaphragmatic aortic balloon-injury followed by high cholesterol diet. At 10 weeks, ce6-mal-alb was administered to 7 atherosclerotic and 7 control animals. 24 hours later, aortic uptake of the ce6-mal-alb uptake was measured *in situ*, using an intravascular fluorescence spectroscopy catheter. Thereafter, the aortas were excised and dissolved in NaOH/SDS for spectrofluorimetric determination of ce6 content.
Results: Intravascular measurements of ce6-mal-alb fluorescence were higher in ATHERO vs. non-athero segments, (8.8±4.8 vs. 2.2±2.2 X10^{3}AU, P<0.001, figure). Further, ce6-mal-alb concentration was higher within the ATHERO aortas (5.2±3.2 vs. 1.9±1.2, ce6 fluorescence/gm tissue x 10^6, p=<0.01). **Conclusion**: Ce6-mal-alb can be employed for intravascular characterization of atherosclerotic plaques. Because this novel PDT compound is selectively toxic to macrophages when light-activated, this agent may be useful for both the detection and therapeutic modification of macrophage-rich plaques.



1200-139 Endogenous Free Radical Generating Sources Are Involved in Smoking-Mediated Dysfunction of Nitric Oxide Biosynthesis in Human Coronary Artery Endothelial Cells: An In Vitro Demonstration

Sudhesh K. Srivastava, Rajat S. Barua, Dhanonjoy C. Saha, Lesley-Jane Eales-Reynolds, Mary C. DeVoe, John A. Ambrose, Saint Vincent Catholic Medical Centers of New York, New York, NY, School of Biomedical and Life Sciences, University of Surrey, Guildford, United Kingdom

Background We have previously demonstrated that cigarette smoking alters NO biosynthesis by reducing NO availability and eNOS activity while increasing eNOS protein expression. Although oxidative stress has been proposed as the leading mechanism for these changes, the source of free radicals is unclear. Cigarette smoke contains high levels of free radicals. However, free radicals potentially arise from endogenous sources as well. This study investigated the role of endogenous free radical generating sources in smoking-related dysfunctional NO biosynthesis in human coronary artery endothelial cells (HCAECs).
Methods and Results Confluent (~85%) monolayers of HCAECs were incubated with serum from 6 nonsmokers and 8 smokers for 12 hours with or without the addition of either 100uM LNMMA, 30uM apocyanin (NADPH oxidase inhibitor), 100uM allopurinol (xanthine oxidase inhibitor) or 2uM rotenone [mitochondrial electron transport chain (METC) inhibitor]. Following incubation, NO levels and eNOS protein expression were measured from the same culture by standard techniques. HCAECs incubated with smokers' serum alone showed significantly lower NO level (0.02±0.01versus 0.13±0.02 uM/pg eNOS/mg total protein, *P*<0.007) and higher eNOS expression (*P*<0.005) compared to nonsmokers. In smokers, only allopurinol (0.04±0.01 uM/pg eNOS/mg total protein) or rotenone (0.06±0.01 uM/pg eNOS/mg total protein) treatment significantly *P*<0.05) improved NO availability. However, levels were still lower compared to nonsmokers (*P*<0.05). Interestingly, when smokers' serum was treated with combined rotenone, allopurinol and 20uM tetrahydrobiopterin (an eNOS cofactor) the NO level, eNOS expression and eNOS activity normalized to that of nonsmokers.
Conclusions To our knowledge this is the first demonstration that endogenous free radical generators such as xanthine oxidase, the METC and eNOS contribute to smoking-mediated dysfunction of NO biosynthesis.

1200-140 Synergistic Effect of Urotensin II With Serotonin on Vascular Smooth Muscle Cell Proliferation

Takuya Watanabe, Takashi Katagiri, Rajbabu Pakala, Claude R. Benedict, Showa University School of Medicine, Tokyo, Japan, University of Texas-Houston Medical School, Houston, TX

Background: Urotensin II (U-II), the most potent vasoconstrictor known to date, and serotonin (5-HT) have been recently shown to play an important role in pulmonary hypertension. However, little is known about the effect of U-II and its interaction with 5-HT on vascular smooth muscle cell (VSMC) proliferation. We assessed the interaction between U-II and 5-HT in inducing VSMC proliferation.
Methods: Growth-arrested rabbit VSMCs were incubated in serum-free medium with different concentrations of U-II and 5-HT. VSMC proliferation was examined by the increase in [3H]thymidine incorporation into cellular DNA and cell number.
Results: U-II or 5-HT induced [3H]thymidine incorporation in a concentration-dependent manner with a maximal effect at a concentration of 50 nanoM (161%) or 50 microM (205%), respectively. When added together, low concentrations of U-II (50 nanoM) and 5-HT (1 microM) interacted synergistically in inducing [3H]thymidine incorporation (382%). These effects on [3H]thymidine incorporation were paralleled by an increase in cell number. The G-protein inactivator GDP-beta-S (100 microM), protein kinase C (PKC) inhibitor Ro31-8220 (0.1 microM), c-Src tyrosine kinase inhibitor PP2 (1 microM), and mitogen-activated protein kinase (MAPK) kinase inhibitor PD098059 (10 microM) significantly inhibited the mitogenic effects of U-II and 5-HT and also their interaction in inducing [3H]thymidine incorporation.
Conclusion: Our results suggest that U-II and 5-HT may induce the synergistic interaction in inducing VSMC proliferation via a G-protein-coupled receptor/PKC/c-Src tyrosine kinase/MAPK pathway, thus contributing to the relatively rapid development of atherosclerosis in hypertensive vascular disease.

1200-141 Systemic Inflammation: Independent Predictor of Contrast Induced Nephropathy

Harish R. Chandra, Peter Kim, James A. Goldstein, Mehrdad H. Sadeghi, William W. O'Neill, William Beaumont Hospital, Royal Oak, MI

Background: Recent observations demonstrate that systemic inflammation may exert adverse influence throughout the vascular bed. This study was designed to determine whether there is a relationship between the presence and magnitude of systemic inflammation and contrast induced nephropathy in patients undergoing coronary angiography.
Methods: In 244 patients undergoing coronary angiography we analyzed the association between systemic markers of inflammation (high sensitivity C-reactive protein, CRP) and contrast induced nephropathy (CIN) defined as decrease in creatinine clearance (CC) of al least 25 cc/min. Univariate and multivariate analysis were performed.
Results: Mean age of the patients was 62±13 yrs and 61% we males. Baseline CC was 89 cc/min. 82% underwent angiography for acute coronary syndrome. 8% developed CIN following the angiography. 2% of the patients in the first tertile of CRP develped CIN compared to 12% in second and 13% in the third tertile of CRP, p=0.03. On multivariate analysis higher CRP tertile was an independent predictor of CIN (OR 2.8, p=0.02) along with use of intra-aortic balloon pump (OR=9.3, p=0.04) and baseline creatinine clearance (OR=1.04, p<0.0001).
Conclusion: These findings demonstrate a strong association between the presence and magnitude of systemic inflammation and development of contrast induced nephropathy.

1200-142 Stabilization of Plaque Size Accompanied by a Time Dependent Decrease in Activated Macrophage Content After Vascular Injury in the Apolipoprotein-E Null Mouse

Christopher J. Davis, Joshua J. Fischer, John M. Sanders, Daniel K. Bennett, Ian J. Sarembock, University of Virginia Health System, Charlottesville, VA

Background: Apolipoprotein-E knockout (ApoE$^{-/-}$) mouse models have been used to study atherosclerosis and responses to vascular injury. Wire injury of the carotid artery results in characteristic neointima formation 28 days after injury. The purpose of this study was to characterize plaque size and cellular content at 2 and 3 months after carotid injury to identify changes in lesion progression.
Methods: Sixteen female ApoE$^{-/-}$ mice were fed a Western diet for one week prior to wire denudation of the left common carotid artery and continued on the atherogenic diet for 4 weeks. After 4 weeks, they were fed a chow diet until sacrifice. Mice were euthanized at 2 months (n=8) and 3 months (n=8) after injury and fasting blood was taken for glucose and cholesterol measurements. Vessels were harvested and paraffin embedded for morphology and immunocytochemistry. The results were compared to 1 month historical controls.
Results: At 1 month, plaque size was 30,500 ± 5,300 μm^2 , macrophage content was 21± 5 %, and cholesterol level was 1170 ± 210 mg/dl. At 2 and 3 months, neointimal areas were not statistically different (35,177 ± 6,789 μm^2 vs. 29,196 ± 7,312μm^2 respectively) and similar to the 1 month group. The presence of activated macrophages in the neointima was significantly higher at 2 months compared to 3 months (26.2 ± 5.7 % vs 4.2 ± 1.5 %, p = .002). There were no significant differences in mean glucose and cholesterol levels at 2 and 3 months (107 ± 7 vs. 108 ± 11 mg/dl and 427 ± 20 vs. 435 ± 35 mg/dl respectively).